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Functional control of gastrin releasing peptide (GRP) mRNA in rat stomach

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The gastric factors controlling abundance of mRNA encoding the important neuropeptide, gastrin releasing peptide (GRP) in rat stomach, were examined by Northern and slot blot analysis. Withdrawal of food increased antral GRP mRNA, as did treatment of fed rats with the acid inhibitory drug, omeprazole. There was no change in GRP mRNA abundance in gastric corpus. The data indicate functionally distinct populations of GRP neurons in different regions of the stomach, and control of antral neuropeptide biosynthesis by the gastric luminal contents.

GRP; Stomach; Acid; Food; mRNA; PCR

1. INTRODUCTION

The processes of gastric digestion are regulated by enteric neurons and gut hormones [1]. Gastrin releasing peptide (GRP), is one of the best studied neuropeptides of the gut: it plays a well recognised role in the control of gastric function as the main neurotransmitter governing release of the important acid-regulatory substances, gastrin and somatostatin [2]. It also has mitogenic effects in vitro and is a potential autocrine growth factor in some tumours [3]. It is now clear that the luminal contents of the stomach, particularly food and acid, modulate the abundance of mRNA species encoding gastrin and somatostatin which are found in epithelial endocrine cells [4–7]. It is not clear, however, whether the luminal contents of the gut are also able to influence synthesis of peptides in enteric neurons. In view of the well-defined physiology of GRP, this peptide provides a good model for examination of this issue. We report here that in a subpopulation of gastric GRP neurons, mRNA abundance is determined by food and acid in the gastric lumen.

2. MATERIALS AND METHODS

2.1. *Animals and treatments*

Studies were made on female Wistar rats of approximately 250 g, kept on a 12-h light/dark cycle. The protocols used to examine the influence of food withdrawal, or of inhibition of gastric acid by the H^+/K^+ ATPase inhibitor, omeprazole, have previously been described [7,8]. In brief, fasted rats were housed on wire-bottomed cages without access to food, but with water ad libitum for 48 h. Omeprazole was given in a dose of 400 μg/kg, p.o., in 0.25% methyl cellulose, daily. At the end of the experimental period rats were killed and the antral and corpus regions of the stomach separately removed for extraction of RNA.

2.2. *Preparation of probes*

Complementary cDNA probes were simultaneously synthesized and radiolabelled by PCR, and purified on Qiagen Tip5 minicolumns as previously described [7,8]. Primers for PCR were synthesized on an Applied Biosystems 391 oligonucleotide synthesizer and corresponded to bases −46 to −26 (sense) and bases 627 to 606 (antisense) of rat GRP cDNA [9]; rat brain cDNA was used as template. Oligo $(dT)_{26}$ was synthesized and end-labelled with polynucleotide kinase according to standard methods. The identity of PCR-generated probes was confirmed by restriction endonuclease digestion and agarose gel electrophoresis (Fig. 1).

2.3. *Preparation and analysis of mRNA*

Total RNA was extracted as previously described [7,8]. Preliminary experiments with Northern blots indicated low message abundance, and subsequent studies were made on mRNA purified on Hybond mAP paper (Amersham) according to the manufacturers instructions. Northern blots were performed as previously described [7,8]. Slot blots were made onto nylon membranes (Hybond N, Amersham) according to the manufacturers protocol, using a Bio-slot manifold (Bio rad). Slot blots were hybridized first with a 672 base pair (bp) PCR-generated cDNA probe for GRP, washed and exposed to Kodak X-AR film according to previously published protocols [7]. The probe was stripped with boiling 0.1% SDS, and the membranes rehybridized with a 362 bp PCR-generated cDNA probe for rat gastrin [7], and finally rehybridized with end-labelled oligo $(dT)_{26}$, to ascertain equal loading. Blots were quantified by video densitometry.

3. RESULTS AND DISCUSSION

Gastric GRP mRNA was present only in low abundance, and in Northern blots of total RNA was scarcely detectable. This is not particularly surprising given that

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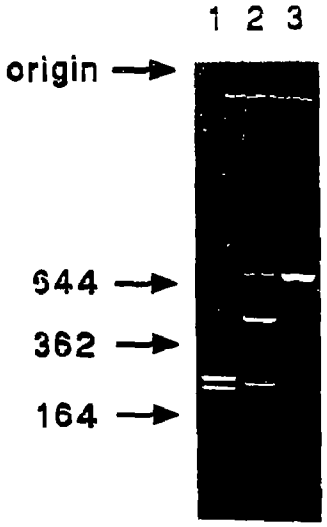

Fig. 1. Restriction analysis of GRP cDNA probe. GRP cDNA was synthesized by PCR as described in Section 2. PCR product (anticipated size 672 bp) was purified by phenol/chloroform extraction, ethanol precipitated and digested with restriction endonuclease. The products were electrophoresed in 1.5% agarose gels. Lane 1, *Pvu*II digest, anticipated products 244, 216 and 129 bp. Lane 2, *Sal*I digest, anticipated products, 445 and 227 bp. Lane 3, *Eco*RI digest, anticipated size 672 bp. Arrows indicate positions of DNA size markers. Note predicted products in each case.

the gene is expressed in a minority of cells of the myenteric plexus, which is itself a very small proportion of total tissue. In Northern blots of gastric mRNA there was a single GRP species of approximately 1 kb, which corresponds to the mRNA previously reported in rat (colon/duodenum) and predicted from the reported cDNA sequence. In brain, an additional 1.5 kb transcript has been described, derived from an alternative

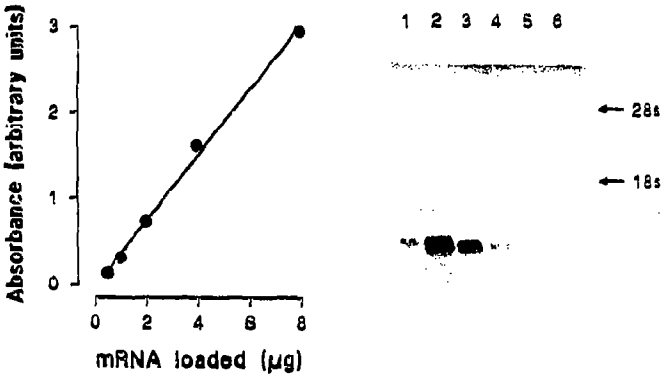

Fig. 2. Relationship between mRNA loaded and signal obtained in Northern blots. Gastric corpus total and mRNA samples were prepared as described in Section 2, electrophoresed in 1% agarose/formaldehyde gels and transferred to nylon membranes. Membranes were hybridized with GRP cDNA probe and quantified by video densitometry. Lane 1, 20 μg total RNA. Lanes 2–6 contain 8, 4, 2, 1 and 0.5 μg mRNA. Size markers are ribosomal subunits. A linear relationship between mRNA loaded and signal was also obtained in slot blots (not shown).

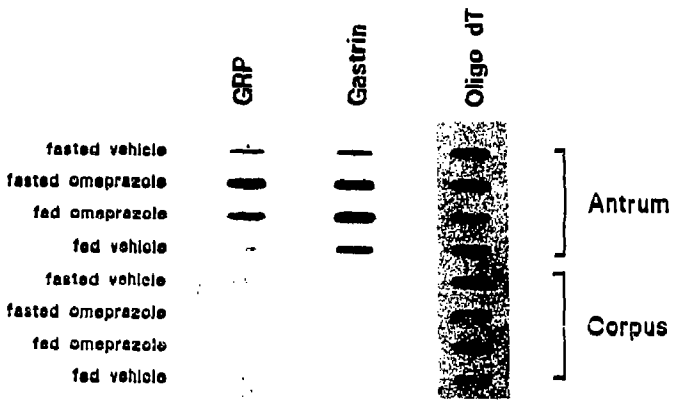

Fig. 3. Slot blot analysis of antral and corpus mRNA from fasted and fed rats treated with omeprazole or vehicle. Membranes were hybridized sequentially with GRP and gastrin cDNA probes, then oligo $(dT)_{26}$. Gastrin and GRP signals were normalized to the oligo (dT) signal.

transcription start site [9]: no evidence of the latter form was found in stomach, either in control tissue or after the various treatments described below, indicating that the alternative start site is not employed in this tissue. There was a linear relationship between signal strength and mRNA loaded, indicating that Northern and slot blots could be used for quantification of GRP mRNA (Fig. 2).

Inhibition of acid secretion with omeprazole in rats fed ad libitum produced a striking 3-fold increase in GRP mRNA in the antral region of the stomach; omeprazole also increased antral GRP mRNA abundance in rats that were fasted for 48 h. It is well established that in these circumstances there is an increase in gastrin

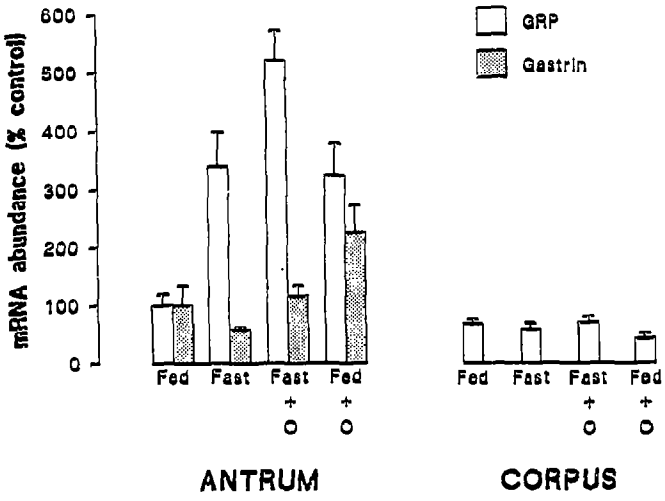

Fig. 4. Gastrin and GRP mRNA abundance in antrum and corpus of fasted and fed rats, treated with omeprazole or vehicle. Values are expressed as percent of antral mRNA levels in fed rats (n=5). No gastrin signal was detected in any corpus mRNA sample. In all three treatment groups, antral GRP mRNA abundance was significantly elevated compared with control animals fed ad libitum (P<0.05, ANOVA).

mRNA [4,5,7], and rehybridization of the membranes with a gastrin probe confirmed the expected increases (Figs. 3 and 4). However, parallel changes in gastrin and GRP mRNAs were not observed after withdrawal of food for 48 h. In this case there was a three-fold increase in antral GRP mRNA, but as expected from previous work [6,7] there was a marked fall in gastrin mRNA. It is of interest that antral somatostatin mRNA abundance increases with food withdrawal, so that changes in GRP mRNA abundance share features in common with both control of somatostatin and gastrin mRNAs. Whether or not the changes in GRP mRNA abundance reflect the response of a single population of neurons remains to be determined. In this context it is important to note, however, that the abundance of GRP mRNA in the acid secreting part of the stomach was not changed by omeprazole or food withdrawal (Figs. 3 and 4). These observations provide prima facie evidence for distinct sub-populations of GRP neurons in the two functionally distinct regions of the stomach; moreover, they suggest that the changes in antral GRP mRNA are not non-specific in nature.

The peptidergic neurons of the gut have been implicated in control of all major aspects of gastrointestinal function including motility, secretion and blood flow [10]. Genes encoding the major neuropeptides have been cloned and sequenced but functional control of peptide production, reflected for example in mRNA abundance, have remained largely unexplored, perhaps in part because mRNA abundance is low. Because GRP has well recognized physiological actions in the stomach, it is a good model for study of this issue. The present data show that mRNA levels for GRP are modulated by luminal contents of the stomach indicating that changes in neuropeptide production depend on food intake. This in turn indicates that altered patterns of turnover of neuropeptide at nerve terminals, in this case controlling gastrin and somatostatin, are likely to be important in adapting gastric mucosal function to changing patterns of luminal stimulation.

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